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09/275,883	03/25/1999	WOLFGANG A. RENNER	1700.0020001	1349

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EXAMINER
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SCHNIZER, RICHARD A

ART UNIT	PAPER NUMBER
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1635

DATE MAILED: 06/17/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

# Office Action Summary

Application No.

09/275,883

Applicant(s)

Renner et al

Examiner

Richard Schnizer

Art Unit

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

## Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

## Status

- 1) ☒ Responsive to communication(s) filed on Mar 31, 2003.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

## Disposition of Claims

- 4) ☒ Claim(s) 75-78, 81-84, 86-103, 105-107, and 109-145 is/are pending in the application.
- 4a) Of the above, claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☒ Claim(s) 102 is/are allowed.
- 6) ☒ Claim(s) 75-78, 81-84, 86-101, 103, 105-107, and 109-145 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claims \_\_\_\_\_ are subject to restriction and/or election requirement.

## Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☒ The drawing(s) filed on Feb 25, 1999 is/are a) ☒ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- 11) ☐ The proposed drawing correction filed on \_\_\_\_\_ is: a) ☐ approved b) ☐ disapproved by the Examiner.
- If approved, corrected drawings are required in reply to this Office action.
- 12) ☐ The oath or declaration is objected to by the Examiner.

## Priority under 35 U.S.C. §§ 119 and 120

- 13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some\* c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
  - ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- \*See the attached detailed Office action for a list of the certified copies not received.
- 14) ☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).
- a) ☐ The translation of the foreign language provisional application has been received.
- 15) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

## Attachment(s)

- 1) ☐ Notice of References Cited (PTO-892) 4) ☐ Interview Summary (PTO-413) Paper No(s). \_\_\_\_\_
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948) 5) ☐ Notice of Informal Patent Application (PTO-152)
- 3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s). \_\_\_\_\_ 6) ☐ Other:

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## **DETAILED ACTION**

### ***Continued Examination Under 37 CFR 1.114***

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 3/31/03 has been entered.

Applicant's amendment after final, received 11/4/02, was entered as Paper No. 25. The amendment was completely responded to in Paper No. 26.

Applicant's Supplemental response containing the Declaration of Dr. Schlesinger, filed 3/31/03 with the request for continued examination, was entered as Paper No. 29.

Claim 79 was canceled and claims 137-145 were added by amendment after final rejection filed 11/4/02.

Claims 75-78, 81-84, 86-103, 105-107, and 109-145 are pending and under consideration in this Office Action.

### ***Claim Rejections - 35 USC § 112***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to

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make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 75-78, 81-84, 86-101, 103, 105-107, and 109-145 are rejected under 35

U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention, for the reasons of record in Paper Nos. 11, 15, and 19.

The claimed invention encompasses the genus of DNA molecules comprising an open reading frame encoding a non-cytopathic, temperature-sensitive alphaviral replicase, wherein the non-cytopathicity and temperature-sensitivity are conferred by one or more mutations in the genes encoding the non-structural proteins of the replicase.

Applicant is referred to the interim guidelines on written description published December 21, 1999 in the Federal Register, Volume 64 Number 244, pp. 71427-71440 (also available at [www.uspto.gov](http://www.uspto.gov)). The following passage is particularly relevant.

The written description requirement for a claimed genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings, or by disclosure of relevant identifying characteristics, i.e. structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between structure and function structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus.

A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within a genus, one must describe a sufficient number of species to reflect the variation within the genus. What constitutes a "representative number" is an inverse function of the skill and knowledge in the art. Satisfactory disclosure of a "representative number" depends on whether one of skill in the art would recognize that applicant was in possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed. In an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus.

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The central issue in this analysis is whether Applicant has disclosed a number of species which is representative of the claimed genus. Applicant discloses a single open reading frame encoding a Sindbis virus RNA-dependent RNA polymerase. This polymerase comprises a P726S nsP2 mutation in combination with a G153E nsP4 mutation. The P726S nsP2 and G153E nsP4 mutations are the structural features which are required to render the Sindbis virus polymerase both temperature sensitive and non-cytopathic. See paragraph bridging pages 21 and 22; page 22, lines 17 and 18; and page 23, lines 22-24. Temperature sensitivity and non-cytopathicity are the necessary common attributes which the polymerase must possess in order to qualify as a member of the claimed genus. However, the specification has failed to disclose what mutations are required to render any other RNA-dependent RNA polymerase both temperature sensitive and non-cytopathic, or what other mutations could confer this phenotype on the Sindbis virus polymerase. The state of the art of the prediction of protein function based on protein structure is not sufficiently advanced to predict *a priori* what mutations will confer temperature sensitivity or non-cytopathicity on a given RNA-dependent RNA polymerase, so it falls to the specification to provide this information. One of skill in the art appreciates that a wide variety of alphaviral RNA-dependent RNA polymerase is known in the art. In view of this recognized variety, and in view of the uncertainty associated with predicting which amino acid substitutions will confer temperature sensitivity and non-cytopathicity on a given polymerase, the disclosure of only a single species is considered insufficient to convey to one of skill in the art that applicant was in possession of the claimed genus at the time of the invention.

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The courts have found that merely describing the functional characteristics of a protein encoded by a particular nucleic acid is insufficient to adequately describe the genus of nucleic acids encoding that protein. A gene is a chemical compound, albeit a complex one, and it is well established in our law that conception of a chemical compound requires that the inventor be able to define it so as to distinguish it from other materials, and to describe how to obtain it. See *Oka*, 849 F.2d at 583, 7 USPQ2d at 1171. Conception does not occur unless one has a mental picture of the structure of the chemical, or is able to define it by its method of preparation, its physical or chemical properties, or whatever characteristics sufficiently distinguish it. It is not sufficient to define it solely by its principal biological property, e.g., non-cytopathic, temperature-sensitive alphaviral replicase, because an alleged conception having no more specificity than that is simply a wish to know the identity of any material with that biological property. When an inventor is unable to envision the detailed constitution of a gene so as to distinguish it from other materials, conception has not been achieved until reduction to practice has occurred, i.e., until after the gene has been isolated. *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016, 1021 (Fed. Cir. 1991). The instant application does not provide a written description that would allow one of skill in the art to immediately envisage the specific structure for Sindbis virus non-cytopathic, temperature-sensitive replicase, or for the broader genus of alphaviral non-cytopathic, temperature-sensitive replicase. *Vas-Cath Inc. v. Mahurkar*, 19USPQ2d 1111, clearly states that "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written

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description' inquiry, *whatever is now claimed* (See page 1117.) The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is claimed" (See *Vas-Cath* at page 1116). As there is no disclosure of the polynucleotides, the skilled artisan cannot envision the detailed chemical structure of the encompassed polynucleotides, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. The nucleic acid itself is required. See *Fiers v. Revel*, 25 USPQ2d 1601 at 1606 (CAFC 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18 USPQ2d 1016.

The limited information provided in the specification is not deemed sufficient to reasonably convey to one skilled in the art that Applicants were in possession of the broadly claimed polynucleotides at the time the application was filed. Thus it is concluded that the written description provision of 35 U.S.C 112, first paragraph, is not satisfied for the claimed polynucleotides. Applicants are reminded that *Vas-Cath* makes clear that the written description provision of 35 U.S.C 112 is severable from its enablement provision (see page 1115).

### ***Response to Arguments***

Applicant's arguments, and the Declaration of Dr. Schlesinger, filed 3/31/03 have been fully considered as they apply to the rejection above but they are not persuasive.

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Applicant considers the issue of written description at pages 3-7 of the response. At page 3, Applicant correctly identifies as the principle inquiry in the written description analysis the issue of whether the disclosed functional characteristics are coupled with a known or disclosed correlation between structure and function. However, Applicant subsequently fails to show any known or disclosed correlation between structure and function that would suffice as an adequate written description of the claimed genus of DNA molecules.

Although Applicant provides evidence of a wide variety of known temperature sensitivity-inducing mutations in non-structural proteins 1-4, no correlation between structure and function is presented such that one of skill in the art could immediately envisage new mutations that would cause temperature sensitivity. Applicant has submitted 11 papers (Exhibits 1-11) published prior to the time the application was filed that disclose temperature sensitive mutations in alphaviral non-structural proteins. Of these eleven publications, only two (Shirako (1990) and Shirako (1998)), disclose mutations made by non-random means. Shirako (1990) teaches that the penultimate amino acids in nsp1 and nsp2 are important for polyprotein processing, and that temperature sensitive mutations in polyprotein processing were known to exist. Shirako then showed that certain mutations in the penultimate amino acid of nsp1 caused temperature sensitive polyprotein processing problems. Shirako (1998) shows that mutations affecting a position at the nsp4 cleavage site can also be temperature sensitive. This is evidence that the art recognized two amino acid of the Sindbis virus non-structural proteins that would rationally be expected to cause temperature sensitive mutations. Applicant has provided no other reasonable correlation between



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structure and function that would allow one to envisage any other temperature sensitive mutations. The findings of the Shirako papers do not explain why one would expect any other mutation not associated with polyprotein cleavage sites to cause temperature sensitive function in the replicase, and the specification fails to provide this information as well. The disclosed mutations do not provide a sufficient correlation between structure and function to allow one to envisage other temperature sensitive mutations, and therefore they do not provide an adequate written description of the genus of temperature sensitive alphaviral replicases.

Even if knowledge of the art was sufficient to adequately describe the genus of temperature sensitive mutations, the written description requirement would still not be met. This is because the claims require more than just temperature sensitivity, the claims also require that the replicase must be non-cytopathic. The specification discloses by structure only a single mutation (P726S in nsp2) that causes a Sindbis alphaviral replicase to be non-cytopathic.

Applicant's response filed 3/31/03 does not disclose any others. So, based on a single mutation in a single structural gene of a single Sindbis alphaviral replicase, Applicant essentially argues that all cytopathic mutations in all genes of all alphaviral replicases have been adequately described, and further that all combinations of non-cytopathic and temperature sensitive mutations that retain the required function have been adequately described. However, the specification provides no further correlation between structure and function that would allow one of skill in the art to envisage any other mutation that causes an alphaviral replicase to be non-cytopathic. No guidance with regard to the particular function of any region of any non-structural protein is provided such that one of

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skill in the art could envisage any single mutation, other than nsp2 P726S, that would cause the required effect. It has been established by the Office that the art of protein structure and function prediction is highly unpredictable. The guidelines on written description published December 21, 1999 in the Federal Register, Volume 64 Number 244, pp. 71427-71440 (also available at [www.uspto.gov](http://www.uspto.gov)) state:

In an unpredictable art, adequate written description of a genus which embraces widely variant species cannot be achieved by disclosing only one species within the genus.

Additionally, the courts in *In re Shokal*, 113 USPQ 283 (CCPA 1957) found that

It appears to be well settled that a single species can rarely, if ever, afford sufficient support for a generic claim. *In re Soll*, 25 C.C.P.A. (Patents) 1309, 97 F.2d 623, 38 USPQ 189; *In re Wahlforss et al.*, 28 C.C.P.A. (Patents) 867, 117 F.2d 270, 48 USPQ 397. The decisions do not however fix any definite number of species which will establish completion of a generic invention and it seems evident therefrom that such number will vary, depending on the circumstances of particular cases. Thus, in the case of small genus such as the halogens, consisting of four species, a reduction to practice of three, or perhaps even two, might serve to complete the generic invention, while in the case of a genus comprising hundreds of species, a considerably larger number of reductions to practice would probably be necessary.

The claimed genus embraces mutations in any of four structurally distinct genes in any known alphavirus. The claims are supported by a description of only a single species. Therefore, one of skill in the art could not conclude that Applicant was in possession of the claimed genus at the time the invention was filed.

The Declaration of Dr. Schlesinger indicates that one of skill in the art understands well how to screen for and obtain temperature sensitive, non-cytopathic alphaviral replicases, without the need to predict the effects of individual or combined mutations on replicase function. The Declaration present no evidence or argument that one of skill in the art appreciates that there was,

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at the time the invention was filed, a sufficient understanding of replicase structure and function to adequately describe the genus of temperature sensitive, non-cytopathic alphaviral replicases. In the absence of such evidence or argument, the Declaration is not considered to overcome the written description rejection.

For these reasons the rejection is maintained.

### *Enablement*

Claims 75-79, 81-84, 86-101, 103, 105-107, and 109-136 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a DNA molecule encoding the Sindbis virus non-cytopathic, temperature-sensitive alphaviral replicase with P726S nsp2 and G153E nsp4 mutations, does not reasonably provide enablement for DNA molecules encoding any other alphaviral non-cytopathic, temperature-sensitive alphaviral replicase. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims for the reasons of record in Paper Nos. 11, 15, 19, 22, and 26.

The claims encompass nucleic acid molecules encoding a non-cytopathic, temperature-sensitive alphaviral replicase, methods of using the nucleic acids, alphaviral particles comprising the nucleic acids, and cells comprising the nucleic acids. The molecules encode an open reading frame which must undergo at least one RNA-dependent RNA polymerase-mediated replication event in order to be translatable.

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As discussed above, the specification discloses only a single example of a non-cytopathic, temperature-sensitive RNA-dependent RNA polymerase, yet the claims encompass the entire genus rather than just the single disclosed species. The prior art teaches several Sindbis virus polymerases which are temperature sensitive. The art teaches several isolates of alphaviruses with non-cytopathic effects, but only a single alphaviral replicase mutation conferring non-cytopathicity has been identified, nsp2 P726S. The scope of the claims is not limited to this single known non-cytopathic Sindbis virus replicase, but embraces all such mutations that could ever occur. The specification fails to provide any guidance as to what amino acids to alter, or in which non-structural protein to alter them, in order to obtain a non-cytopathic replicase other than one comprising nsp2 P726S. The specification fails to disclose any example, other than that encoded by SEQ ID NO:1, of a polymerase which is both temperature sensitive and non-cytopathic. Further, while it is simple to construct nucleic acids which would comprise both types of mutations, the characteristics of these novel polypeptides would be highly unpredictable, as stated in the previous office actions. The reason for this is that it is not currently possible to accurately predict the effects of mutations on the function of proteins. For example, Rudinger (In peptide Hormones, J.A. Parsons Ed. University Park press, Baltimore, 6/1996) teaches that "[t]he significance of particular amino acids and sequences for different aspects of biological activity cannot be predicted a priori but must be determined from case to case by painstaking experimental study." See page 6, last paragraph. Furthermore, Schnizer et al (Arch Biochem Biophys. 1996 Feb 1;326(1):126-36) teach an example in which mutations of two separate amino acids of the

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yeast F1-ATPase beta subunit were combined and produced totally unpredictable results.

Specifically, one mutation at position 203 and five different mutations at position 211 were found to inactivate and destabilize the F1-ATPase complex when expressed separately. However, when the position 203 mutation was combined with any one of the position 211 mutations in the same construct, destabilization was suppressed and activity was restored to the ATPase complex.

See abstract. While this result may allow certain conclusions to be drawn about structural and functional relationships within the ATPase, it could not have been predicted *a priori*. Similarly the effects of combining mutation in the Sindbis virus polymerases cannot be predicted *a priori*. One might argue that it would not be undue experimentation to express and assay each construct individually and thereby determine empirically which ones encoded polymerases of the desired phenotype. However, as set forth in *In re Fisher*, 166 USPQ 18 (CCPA 1970), compliance with 35 USC 11 11 2, first paragraph requires:

that scope of claims must bear a reasonable correlation to scope of enablement provided by the specification to persons of ordinary skill in the art; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, the scope of enablement varies inversely with degree of unpredictability of factors involved.

In this case, the art is not sufficiently advanced to allow the prediction of mutations that will cause non-cytopathicity, or to allow the prediction of the effects of combining temperature-sensitive and non-cytopathic mutations. Furthermore, Applicant has disclosed mutations only of a Sindbis virus replicase, whereas the claims encompass replicases from all alphaviruses. One of skill in the art

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could not predict which, if any, of these replicases could be mutated to be appropriately temperature sensitive and non-cytopathic, or what mutations would be required for this.

In view of the unpredictability of polypeptide structure-function relationships, the failure of the specification to disclose more than one example of a Sindbis virus temperature sensitive, non-cytopathic RNA-dependent RNA polymerase, and the failure of the specification to provide any guidance as to what mutations other than nsp2 P726S will provide a non-cytopathic replicase, one of skill in the art could not make the invention commensurate in scope with the claims.

### ***Response to Arguments***

Applicant's arguments, and the Declaration of Dr. Schlesinger, filed 3/31/03 have been fully considered as they apply to the rejection above but they are not persuasive.

Applicant considers the enablement rejection at pages 7-9 of the response. At pages 7 and 8 of the response, Applicant reiterates arguments made in the replies filed 11/4/02, and addressed in the Advisory Action of 11/14/02. Applicant relies for further support on currently filed exhibits 2, 4, 9, and 12, which provide evidence that one of skill in the art knows how to screen for and detect temperature sensitive alphaviral replicase mutants and that the single non-cytopathic alphaviral replicase substitution mutation disclosed in the application was known in the prior art. Additionally, Applicant relies on the Declaration of Dr. Schlesinger as evidence that one of skill in the art at the time of the invention could have obtained temperature sensitive and non-cytopathic

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alphaviral replicases without the need to predict effects of individual or combined mutations on replicase functions.

The Examiner has not contested that one could screen for and obtain temperature sensitive mutants. The issues in the rejection were whether one could make without undue experimentation DNA encoding alphaviral replicases comprising non-cytopathicity mutations other than nsp2 P726S, and whether one could make without undue experimentation DNA encoding alphaviral replicases, other than SEQ ID NO:1, that were both temperature sensitive and non-cytopathic. The scope of the claims is not limited to the single known non-cytopathic Sindbis virus replicase, but embraces all such mutations that could ever occur. The specification fails to provide any guidance as to what amino acids to alter, or in which non-structural protein to alter them, in order to obtain a non-cytopathic replicase other than one comprising nsp2 P726S. Furthermore, the specification provides no guidance as to how to predict the effects on alphaviral activity of combining temperature sensitive and non-cytopathic mutations. Applicant's response provided no evidentiary basis to overcome the rejection by either showing that the art was predictable, or by showing that trial and error experimentation in the absence of guiding principles of structure and function would be required. Regarding the Declaration of Dr. Schlesinger, the MPEP 2164.05 state in part: "Applicant may submit factual affidavits under 37 CFR 1.132 or cite references to show what one skilled in the art knew at the time of filing the application. A declaration or affidavit is, itself, evidence that must be considered. **The weight to give a declaration or affidavit will depend upon the amount of factual evidence the declaration or**

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**affidavit contains to support the conclusion of enablement.** In re Buchner, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991) (“expert’s opinion on the ultimate legal conclusion must be supported by something more than a conclusory statement”). Emphasis added. In this case the Declaration provides no factual evidence whatsoever, and is only a statement of Declarants beliefs. As such it is insufficient to overcome the prima facie case established by the Office. For these reasons the rejection is maintained.

DAVE T. NGUYEN  
PRIMARY EXAMINER



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***Conclusion***

Claim 102 is allowable.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Richard Schnizer, whose telephone number is 703-306-5441. The examiner can normally be reached Monday through Friday between the hours of 6:20 AM and 3:50 PM. The examiner is off on alternate Fridays, but is sometimes in the office anyway.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, John Leguyader, can be reached at 703-308-0447. The FAX numbers for art unit 1632 are 703-308-4242, and 703-305-3014. Additionally correspondence can be transmitted to the following RIGHTFAX numbers: 703-872-9306 for correspondence before final rejection, and 703-872-9307 for correspondence after final rejection.

Inquiries of a general nature or relating to the status of the application should be directed to the Patent Analyst Trina Turner whose telephone number is 703-305-3413.

Richard Schnizer, Ph.D.

  
DAVE T. NGUYEN  
PRIMARY EXAMINER